Talanta 130 (2014) 382–387

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Short communication

Bio-inspired solid phase extraction sorbent material for cocaine: A cross reactivity study

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ARTICLE INFO

Article history:
Received 29 March 2014
Received in revised form
6 July 2014
Accepted 7 July 2014
Available online 15 July 2014

Keywords:
Peptides
Cocaine
Molecular modeling
Solid phase extraction
Cross reactivity
Liquid chromatography–mass spectrometry

ABSTRACT

The binding specificity of a bio-inspired hexapeptide (QHWWDW) versus cocaine and four other drugs such as 3,4-methylenedioxy-N-methylamphetamine (MDMA), 3,4-methylenedioxy-N-ethylamphetamine (MDEA), phencyclidine and morphine was computationally studied and then experimentally confirmed in solid phase extraction (SPE) followed by liquid chromatography–mass spectrometry (LC/MS) detection. In simulation, the hexapeptide-drug complexes were docked with different scoring functions and considering pH chemical environment. In experimental, the cross reactivity of the selected hexapeptide was tested as SPE sorbent versus cocaine and other four drugs using buffer solutions at pH 4 and 7. Significant differences in specific retention were found between cocaine (97% of recovery) and both morphine (45% of recovery) and phencyclidine (60% of recovery), but less for ecstasies (average recovery 69%). In agreement with docking simulation, the hexapeptide showed the highest recovery with best specificity versus cocaine at pH 7 with an experimentally binding constant of $2.9 \times 10^6\ M^{-1}$. The bio-inspired sorbent material analytical performances were compared with a commercial reversed phase cartridge confirming the hexapeptide specificity to cocaine and validating simulated data.

1. Introduction

Cocaine is a well-known sympathomimetic drug representing a considerable health-emergency for publics [1,2]. This illicit drug and its metabolites are more and more considered as the latest group of emerging environmental pollutants [3,4] and the identification of non-approved drugs is a great challenge for control laboratories. The robustness of methods and techniques for identification and discrimination of illegal and legal drugs of abuse has been investigated in different works [5,6].

Analytical and bio-analytical methods were proposed for drug monitoring and detection in samples [7–9]. The most explored method is, in different experimental design configurations, the use of solid phase extraction followed by chromatography–mass spectrometry [9–13]. In general, there is a great interest in the development of pre-analytical tools for clean-up and pre-concentration of analytes [14–16]. In fact, sorbent materials are often not selective and can result in the co-elution of interfering compounds with similar polarity, affecting the reliability of the analytical methods. To overcome this issue, different approaches have been studied in order to produce specific affinity-based stationary phases. To this end, selective ligands such as molecularly imprinted polymers, aptamers or peptides have been developed, offering a viable and cost-effective alternative to antibodies which are expensive and challenging to prepare [14,17–20]. These engineered receptors are designed to target specific molecules, similarly to enzymes or biological receptors. They usually show lower affinities but they also offer some advantages such as low costs, rapid synthesis and stability.

In this work, the application of a bio-inspired molecularly modeled peptide was proved to be a selective sorbent material for cocaine vs. ecstasies, phencyclidine and morphine. The experimental data was supported by a molecular docking procedure. Over the last decade molecular docking has demonstrated its usefulness, in areas such as the identification of lead compounds and drug discovery [21–23]. This approach has been very often used in combination with the implementation of consensus scoring [24], considerations on both ligand and receptor flexibility [25], and the inclusion of semi-empirical and/or molecular mechanics methods for the assessment of the binding energies [26–29]. The simulated

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http://dx.doi.org/10.1016/j.talanta.2014.07.017
0039-9140/

conformations of receptors when interacting with their target molecules have demonstrated their potential and predictive capabilities for subsequent development of experimental methods [20,30–35]. An important goal in molecular design is to collect several datasets for the improvement of docking and scoring methods [10,36,37]. In particular, the modification of basic parameters in molecular modeling software was shown to have a significant effect on docking and virtual screening results [38–42].

This work shows how molecular modeling method can be used as a convenient tool for the optimization of SPE conditions. The key point in optimizing experimental cocaine specificity lies on considering the effect of the pH. This was calculated here by changing protonation states of histidine. Moreover, the orientation of the complexes within the binding site, outputted by the docking software, was very useful in experimental strategy design.

The introduction of predictive computational models in analytical protocols, instead of trial and error procedures, offers advantages in minimizing experimental problems currently encountered, such as non-specific recognition, reagent stability and separation procedures.

2. Materials and methods

2.1. Virtual screening process

A desktop PC with a 3.4 GHz Intel Core I7-2600 processor, 8 GBytes DDR3 RAM with 1333 MHz bus, running Microsoft Windows 7 Professional 64 Bits was used for the entire screening process, molecular modeling experiments, post-calculations and data analysis.

The virtual screening process was carried out using an automated pipeline of computational tools bundled in OpenEye Scientific Software package under academic license. The automation was achieved with AutoIT V3, a freeware BASIC-like scripting language. A database of ligands was generated by converting standard IUPAC names into structures using LEXICHEM package [43]. Each of these structures was subsequently optimized using molecular mechanics as implemented in SZYBKI 1.5.7 in its default parameterization [44]. In order to contemplate molecules flexibility, for both receptor and ligands a set of conformers was generated with OMEGA 2.4.6 [45,46]. An exhaustive rigid body docking was implemented using FRED 2.2.5 and 3.0.0 [47]. The visualization of molecular structures for pre- and post-processing and analysis was carried out with VIDA 4.2.1 [48].

The hexapeptide QHWWDW was designed in zwitterion form with one or two NH bonds on the imidazole ring, aiming to simulate its behavior at pH 7 and pH 4-. The net charge at pH 7, its isoelectric point and hydropathy index were calculated using a peptide calculator [49].

During the docking process, the entire surface of each hexapeptide conformer was considered suitable to form positive interactions with ligand molecules. In consequence, a box, defining the docking active site, was generated for each conformer encapsulating the entire peptide, with sizes comprised from 4500 $Å^3$ to 7200 $Å^3$. The required time to process each conformer, from the initial design to final docking stage, was about 1 min.

FRED 3.0.0 was run only in its default parameterization with Chemgauss4 function, a modification of Chemgauss3 (Ch3), with improved hydrogen bonding and metal chelator terms. Instead, with FRED 2.2.5, three parameters were tested: 1-poses ranking through exhaustive scoring; 2-systematic solid body optimization functions; and 3- consensus structure score evaluation.

The default solid body optimization function in FRED 2.2.5 was Ch3, but also, Chemgauss2 (Ch2) and Shapegauss (Sh) functions were tested. Functions PLP and CGO were not considered in this work because they gave no effective poses.

The available alternatives to FRED 2.2.5 default scoring function Ch3 were Sh, PLP, CGO, CGT Ch2, Ch3, Chemscore (Cs), OEChemscore (Ocs), Screenscore (Ss), or none (Nn), each one of them presenting a particular combination of speed and atomic interactions awareness. CGO and CGT were not suitable in this type of simulation experiment, therefore, when tested, both functions generated errors and were discarded.

By default FRED 2.2.5 used a consensus of multiple scoring functions to rank one ligand against another. This consensus score was calculated based on the combined results of PLP, Ch3 and Ocs. However, different combinations of other scoring functions available (Sh, PLP, CGO, CGT Ch2, Ch3, Cs, Ocs, Ss and Zapbind) were also used in this study. Consensus scoring failed when using functions PLP, Zapbind, CGO and CGT. These errors occurred due to internal unexpected miscalculations in the software, atom types mismatch, and other factors.

The scoring function was given by the sum of different terms like shape, hydrogen bond, aromatic, desolvation and others. The major difference in scoring functions was the use or exclusion of these terms in calculating the score. None of the functions had intramolecular terms.

3. Extraction procedure and liquid chromatography–mass spectrometry analysis (LC–MS/MS)

3.1. Chemicals

Standards of cocaine (COC), 3,4-methylenedioxy-N-methylamphetamine (MDMA), 3,4-methylenedioxy-N-ethylamphetamine (MDEA), phencyclidine (PCP) and morphine (MOR) were purchased from LGC Standard (Italy). The purity of the reference compounds was $\geq$ 99%. All standards were provided at a concentration of 3 mM. Individual stock solutions were prepared in methanol at 300 μM and working standard mixtures were prepared by appropriate dilution of the standard solutions in methanol. All solutions were stored at -20 °C in dark condition. Acetonitrile and methanol were of RS-Plus grade. Ultrapure water was produced by a Milli-Q Plus apparatus from Millipore (USA). Acetonitrile and methanol were of RS-Plus grade. All reagents used for the preparation of aqueous buffers, were purchased from Carlo Erba (Italy).

The solid phase extraction sorbent material QHWWDW-resin (Nova Syn TGA), with a peptide substitution level of 0.17 mmol g^{-1} was synthetized by EspiKem srl (Italy). Strata-X 33 μm polymeric reversed phase cartridges (30 mg/mL) were from Phenomenex. SPE Isolute column (Empty 1 mL Reservoir) was from STEPBIO (Italy).

3.2. Extraction procedure

The cartridges (volume 1 ml) were packed with 30 mg of resin (the blank) or modified peptide resin dissolved in 5 mL of an ethanol/water solution (80:20, v/v) and kept at room temperature for 6–8 h. This suspension was slowly loaded into the cartridge with a teflon frit on the bottom. During this procedure, the cartridge was continuously shaken in order to obtain a homogeneous packing. After loading, a second frit was used to cover the resin into the cartridge. Then the cartridge was conditioned and equilibrated by washing with ethanol. All the experiments were carried out by means of a VISIPREP device and the solvent fractions collected were named progressively.

The extraction procedure was performed in four steps:

1. Conditioning of the stationary phase with Tris–HCl (pH=7).
2. Sample loading (1 mL).